

# E.Z.N.A.® MicroElute Genomic DNA Kit

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# Introduction

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The E.Z.N.A.® MicroElute® Genomic DNA Kit provides a rapid and easy method for the isolation of genomic and mitochondrial DNA from small sample sizes or from a large volume of samples for consistent PCR and other downstream applications. This kit can be used for the isolation of genomic DNA from micro-dissected tissue, cultured cells, blood, dry blood, swabs, buffy coat, serum, urine and plasma. The kit allows single or multiple processing of samples. There is no need for phenol/chloroform extractions and time-consuming steps such as precipitation with isopropanol or ethanol are eliminated.

The E.Z.N.A.® MicroElute® Genomic DNA Kit uses the reversible binding properties of the HiBind® matrix, a silica-based material, in combination with the MicroElute® spin column technology to allow elution volume as small as 10 µL. A specially formulated buffer system allows genomic DNA up to 40 kb to bind to the matrix. Samples are lysed under denaturing conditions and then transferred to the MicroElute® DNA Mini Columns to bind DNA. Cellular debris, hemoglobin, and other proteins are efficiently eliminated via three quick wash steps. High-quality DNA is eluted in sterile deionized water or low salt buffer.

## **New in this Edition:**

- HB Buffer has been replaced by HBC Buffer. Isopropanol is required and supplied by the user.
- Equilibration Buffer is no longer included with this kit. An optional Column Equilibration Protocol has been added to the protocol for your convenience.
- Equilibration Buffer is replaced with 3M NaOH provided by the user.

# Kit Contents

Product	D3096-00	D3096-01	D3096-02
Purifications	5	50	200
MicroElute® DNA Mini Columns	5	50	200
2 mL Collection Tubes	10	100	400
BL Buffer	5 mL	35 mL	125 mL
TL Buffer	5 mL	35 mL	125 mL
HBC Buffer	3 mL	25 mL	80 mL
OB Protease Solution	120 µL	1.1 mL	4 x 1.1 mL
Linear Acrylamide (5 mg/mL)	25 µL	125 µL	500 µL
DNA Wash Buffer	2 mL	15 mL	3 x 20 mL
Elution Buffer	5 mL	10 mL	30 mL
User Manual	✓	✓	✓

## Storage and Stability

All of the E.Z.N.A.® MicroElute® Genomic DNA Kit components are guaranteed for at least 24 months from the date of purchase when stored as follows. OB Protease Solution can be stored at room temperature for up to 12 months. For long-term storage, store OB Protease Solution at 2-8°C. During shipment or storage in cool ambient conditions, precipitates may form in BL Buffer. Dissolve such deposits by warming the solution at 37°C and gently shaking.

## Determination of DNA Quality and Quantity

The total DNA yield can be determined by a spectrophotometer using deionized water, Tris-HCl buffer, or Elution Buffer as blank. DNA concentration is calculated as:

$$[\text{DNA}] = (\text{Absorbance}_{260}) \times (0.05 \mu\text{g}/\mu\text{L}) \times (\text{Dilution factor})$$

The quality of DNA can be assessed by measuring absorbance at both 260 nm and at 280 nm. A ratio of ( $A_{260}/A_{280}$ ) of 1.7-1.9 corresponds to 85%-95% purity. Yields vary with both amount and type of sample used.

# Preparing Reagents

1. Dilute DNA Wash Buffer with 100% ethanol as follows and store at room temperature.

Kit	100% Ethanol to be Added
D3096-00	8 mL
D3096-01	60 mL
D3096-02	80 mL per bottle

2. Dilute HBC Buffer with isopropanol as follows and store at room temperature.

Kit	Isopropanol to be Added
D3096-00	1.2 mL
D3096-01	10 mL
D3096-02	32 mL

# Protocol for Tissue Samples

## E.Z.N.A.<sup>®</sup> MicroElute<sup>®</sup> Genomic DNA Kit - Protocol for Tissue Samples

The following protocol allows for isolation of genomic DNA from up to 10 mg tissue. Yields will vary depending on source. To purify very small amounts of DNA from a sample, such as low volumes of blood (<10  $\mu$ L) or micro-dissected tissues, we recommend adding Linear Acrylamide to BL Buffer to enhance the DNA binding ability of the column. In most cases, adding 5-10  $\mu$ g (1-2  $\mu$ L) per sample is sufficient.

### Materials and Equipment to be Supplied by User:

- Microcentrifuge capable of at least 13,000 x g
- Nuclease-free 1.5 mL or 2 mL microcentrifuge tubes
- Water baths, heat blocks, or incubators capable of 55°C and 70°C
- Shaking water bath capable of 55°C
- Vortexer
- 100% ethanol
- Isopropanol
- Optional: RNase A (25 mg/mL)
- Optional: sterile deionized water
- Optional: 3M NaOH

### Before Starting:

- Prepare DNA Wash Buffer and HBC Buffer according to the "Preparing Reagents" section on Page 4
- Set water baths, heat blocks, or incubators to 55°C and 70°C
- Heat Elution Buffer to 70°C

1. Mince up to 10 mg tissue and transfer to a 1.5 mL microcentrifuge tube (not provided).
2. Add 200  $\mu$ L TL Buffer
3. Add 20  $\mu$ L OB Protease Solution. Vortex to mix thoroughly.
4. Incubate at 55°C in a shaking water bath.

**Note:** If a shaking water bath is not available, vortex the sample every 10-20 minutes. Lysis time will depend on the amount and type of tissue, but is usually less than 3 hours. Lysis can proceed overnight.

# Protocol for Tissue Samples

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5. Centrifuge at maximum speed (13,000 x g) for 2 minutes.
6. Transfer the supernatant to a clean 1.5 mL microcentrifuge tube (not provided).

**Optional:** Certain tissues such as liver have high levels of RNA which will be co-purified with DNA using this kit. While it will not interfere with PCR, the RNA may be removed at this point. Add 5  $\mu$ L RNase A (25 mg/mL, assuming a sample size of 10 mg) and incubate at room temperature for 2 minutes. Proceed to Step 7.

7. Add 220  $\mu$ L BL Buffer. Vortex to mix thoroughly.

**Note:** If Linear Acrylamide is needed, add 1-2  $\mu$ L Linear Acrylamide to 220  $\mu$ L BL Buffer.

8. Incubate at 70°C for 10 minutes.
9. Add 220  $\mu$ L 100% ethanol. Vortex at maximum speed for 15 seconds. A precipitate may form at this point; it will not interfere with DNA isolation.
10. Briefly centrifuge to bring down any liquid from the top of the lid.
11. Insert a MicroElute® DNA Mini Column into a 2 mL Collection Tube.

## Optional Protocol for Column Equilibration:

1. Add 100  $\mu$ L 3M NaOH to the MicroElute® DNA Mini Column.
  2. Centrifuge at maximum speed for 30-60 seconds.
  3. Discard the filtrate and reuse the collection tube.
12. Transfer the sample from Step 10 (including any precipitate that may have formed) to the MicroElute® DNA Mini Column.
  13. Centrifuge at maximum speed for 1 minute.
  14. Discard the filtrate and the Collection Tube.

# Protocol for Tissue Samples

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15. Transfer the MicroElute® DNA Mini Column to a new 2 mL Collection Tube.

16. Add 500 µL HBC Buffer.

**Note:** HBC Buffer must be diluted with isopropanol before use. Please see Page 4 for instructions.

17. Centrifuge at maximum speed for 30 seconds.

18. Discard the filtrate and reuse the Collection Tube.

19. Add 700 µL DNA Wash Buffer.

**Note:** DNA Wash Buffer must be diluted with 100% ethanol prior to use. Please see the Preparing Reagents section on Page 4 for instructions.

20. Centrifuge at maximum speed for 1 minute.

21. Discard the filtrate and reuse the Collection Tube.

22. Repeat Steps 19-21 for a second DNA Wash Buffer wash step.

23. Centrifuge the empty MicroElute® DNA Mini Column at maximum speed for 2 minutes to dry the membrane.

**Note:** It is important to dry the column membrane before elution. Residual ethanol may interfere with downstream applications.

24. Transfer the MicroElute® DNA Mini Column to a nuclease-free 1.5 or 2 mL microcentrifuge tube (not provided).

25. Add 10-50 µL Elution Buffer (or sterile deionized water) heated to 70°C.

**Note:** Smaller elution volumes will increase DNA concentration but decrease yield. Elution volumes greater than 200 µL are not recommended.

# Protocol for Tissue Samples

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26. Let sit at room temperature for 3 minutes.
27. Centrifuge at maximum speed for 1 minute.
28. Store DNA at  $-20^{\circ}\text{C}$ .



# Protocol for Small Volumes of Blood, Serum, or Fluids

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## E.Z.N.A.<sup>®</sup> MicroElute<sup>®</sup> Genomic DNA Kit - Protocol for Small Volumes of Blood, Serum, or Fluids

This protocol is designed for the rapid isolation of DNA from 1-100  $\mu\text{L}$  blood (treated with EDTA, citrate, or heparin-based anticoagulants), serum, plasma, buffy coat, saliva, and urine. To purify very small amounts of DNA from a sample, such as low volumes of blood ( $<10 \mu\text{L}$ ) or micro-dissected tissues, we recommend adding Linear Acrylamide to BL Buffer to enhance the DNA binding ability of the column. In most cases, adding 5-10  $\mu\text{g}$  (1-2  $\mu\text{L}$ ) per sample is sufficient.

### Materials and Equipment to be Supplied by User:

- Microcentrifuge capable of at least 13,000  $\times g$
- Nuclease-free 1.5 mL or 2 mL microcentrifuge tubes
- Water bath, heat block, or incubator capable of 70°C
- Vortexer
- Isopropanol
- 100% ethanol
- PBS
- Optional: sterile deionized water
- Optional: 3M NaOH

### Before Starting:

- Prepare DNA Wash Buffer and HBC Buffer according to the "Preparing Reagents" section on Page 4
- Set water bath, heat block, or incubator to 70°C
- Heat Elution Buffer to 70°C

1. Add 1-100  $\mu\text{L}$  sample (the sample must be at room temperature) to a 1.5 mL microcentrifuge tube (not provided).
2. Adjust the sample volume to 100  $\mu\text{L}$  with PBS.
3. Add 20  $\mu\text{L}$  OB Protease solution. Vortex to mix thoroughly.
4. Add 120  $\mu\text{L}$  BL Buffer. Vortex to mix thoroughly.

# Protocol for Small Volumes of Blood, Serum, or Fluids

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5. Incubate at 70°C for 10 minutes.
6. Add 160  $\mu$ L isopropanol. Vortex at maximum speed for 15 seconds. A precipitate may form at this point; it will not interfere with DNA isolation.
7. Briefly centrifuge to bring down any liquid from the top of the lid.
8. Insert a MicroElute® DNA Mini Column into a 2 mL Collection Tube.

## Optional Protocol for Column Equilibration:

1. Add 100  $\mu$ L 3M NaOH to the MicroElute® DNA Mini Column.
  2. Centrifuge at maximum speed for 30-60 seconds.
  3. Discard the filtrate and reuse the collection tube.
9. Transfer the sample from Step 7 (including any precipitate that may have formed) to the MicroElute® DNA Mini Column.
  10. Centrifuge at maximum speed for 1 minute.
  11. Discard the filtrate and the Collection Tube.
  12. Transfer the MicroElute® DNA Mini Column to a new 2 mL Collection Tube.
  13. Add 500  $\mu$ L HBC Buffer.  
**Note:** HBC Buffer must be diluted with isopropanol before use. Please see Page 6 for instructions.
  14. Centrifuge at maximum speed for 30 seconds.
  15. Discard the filtrate and reuse the Collection Tube.

# Protocol for Small Volumes of Blood, Serum, or Fluids

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16. Add 700  $\mu$ L DNA Wash Buffer.

**Note:** DNA Wash Buffer must be diluted with 100% ethanol prior to use. Please see the Preparing Reagents section on Page 4 for instructions.

17. Centrifuge at maximum speed for 1 minute.

18. Discard the filtrate and reuse the Collection Tube.

19. Repeat Steps 16-18 for a second DNA Wash Buffer wash step.

20. Centrifuge the empty MicroElute<sup>®</sup> DNA Mini Column at maximum speed for 2 minutes to dry the membrane.

**Note:** It is important to dry the column membrane before elution. Residual ethanol may interfere with downstream applications.

21. Transfer the MicroElute<sup>®</sup> DNA Mini Column to a nuclease-free 1.5 or 2 mL microcentrifuge tube (not provided).

22. Add 10-50  $\mu$ L Elution Buffer (or sterile deionized water) heated to 70°C.

**Note:** Smaller elution volumes will increase DNA concentration but decrease yield. Elution volumes greater than 200  $\mu$ L are not recommended.

23. Let sit at room temperature for 3 minutes.

24. Centrifuge at maximum speed for 1 minute.

25. Store DNA at -20°C.

# Protocol for Dried Blood, Fluids, and Semen Spots

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## E.Z.N.A.® MicroElute® Genomic DNA Kit - Protocol for Dried Blood, Fluids, and Semen Spots

Dried blood, body fluids, and semen samples on filter paper can be processed using the following method.

### Materials and Equipment to be Supplied by User:

- Microcentrifuge capable of at least 13,000 x *g*
- Nuclease-free 1.5 mL or 2 mL microcentrifuge tubes
- Water baths, heat blocks, or incubators capable of 55°C and 70°C
- Shaking water bath capable of 55°C
- Vortexer
- 100% ethanol
- Isopropanol
- Optional: sterile deionized water
- Optional: Dithiothreitol (DTT)
- Optional: 3M NaOH

### Before Starting:

- Prepare DNA Wash Buffer and HBC Buffer according to the “Preparing Reagents” section on Page 4
- Set water baths, heat blocks, or incubators to 55°C and 70°C
- Heat Elution Buffer to 70°C

1. Cut or punch out the blood (or other sample) spot from the filter paper. Tear or cut the filter paper into small pieces and place into a 1.5 mL microcentrifuge tube (not provided).

**Note:** Use 1-3 punched circles (~3 mm diameter) for each DNA isolation.

2. Add 200 µL TL Buffer and 20 µL OB Protease Solution. If you are processing semen spots, add 20 µL DTT to each sample.
3. Incubate at 55°C for 45-60 minutes in a shaking water bath.

**Note:** If a shaking water bath is not available, vortex the sample every 10-20 minutes.

# Protocol for Dried Blood, Fluids, and Semen Spots

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4. Briefly centrifuge to bring down any liquid from the top of the tube.
5. Add 220  $\mu$ L BL Buffer. Vortex to mix thoroughly.  
**Note:** If only one punch card is processed, add 1  $\mu$ L Linear Acrylamide to the sample.
6. Incubate at 70°C for 10 minutes. Vortex at maximum speed for 10 seconds several times during incubation.
7. Centrifuge at maximum speed for 5 minutes.
8. Transfer the cleared lysate to a new 1.5 mL microcentrifuge tube (not provided).  
**Note:** For maximum yield collect any remaining liquid from the paper and transfer the entire sample, including paper, into an Omega Homogenizer Column (not provided, Cat# HCR003) and centrifuge at maximum speed for 2 minutes to collect all of the lysate.
9. Add 220  $\mu$ L 100% ethanol. Vortex at maximum speed for 15 seconds.
10. Briefly centrifuge to bring down any liquid from the top of the lid.
11. Insert a MicroElute® DNA Mini Column into a 2 mL Collection Tube.

## Optional Protocol for Column Equilibration:

1. Add 100  $\mu$ L 3M NaOH to the MicroElute® DNA Mini Column.
  2. Centrifuge at maximum speed for 30-60 seconds.
  3. Discard the filtrate and reuse the collection tube.
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12. Transfer the sample from Step 10 (including any precipitate that may have formed) to the MicroElute® DNA Mini Column.
  13. Centrifuge at maximum speed for 1 minute.

# Protocol for Dried Blood, Fluids, and Semen Spots

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14. Discard the filtrate and the Collection Tube.
15. Transfer the MicroElute® DNA Mini Column to a new 2 mL Collection Tube.
16. Add 500 µL HBC Buffer.  
**Note:** HBC Buffer must be diluted with isopropanol before use. Please see Page 6 for instructions.
17. Centrifuge at maximum speed for 30 seconds.
18. Discard the filtrate and reuse the Collection Tube.
19. Add 700 µL DNA Wash Buffer.  
**Note:** DNA Wash Buffer must be diluted with 100% ethanol prior to use. Please see the Preparing Reagents section on Page 4 for instructions.
20. Centrifuge at maximum speed for 1 minute.
21. Discard the filtrate and reuse the Collection Tube.
22. Repeat Steps 19-21 for a second DNA Wash Buffer wash step.
23. Centrifuge the empty MicroElute® DNA Mini Column at maximum speed for 2 minutes to dry the membrane.  
**Note:** It is important to dry the column membrane before elution. Residual ethanol may interfere with downstream applications.
24. Transfer the MicroElute® DNA Mini Column to a nuclease-free 1.5 or 2 mL microcentrifuge tube (not provided).
25. Add 10-50 µL Elution Buffer (or sterile deionized water) heated to 70°C.

## Protocol for Dried Blood, Fluids, and Semen Spots

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**Note:** Smaller elution volumes will increase DNA concentration but decrease yield. Elution volumes greater than 200  $\mu\text{L}$  are not recommended.

26. Let sit at room temperature for 3 minutes.
27. Centrifuge at maximum speed for 1 minute.
28. Store DNA at  $-20^{\circ}\text{C}$ .

# Protocol for Swabs

## E.Z.N.A.<sup>®</sup> MicroElute<sup>®</sup> Genomic DNA Kit - Protocol for Swabs

This protocol is designed for the isolation of genomic DNA from semen swabs, blood swabs, and buccal swabs.

### Materials and Equipment to be Supplied by User:

- Microcentrifuge capable of at least 13,000 x *g*
- Nuclease-free 2 mL microcentrifuge tubes
- Water baths, heat blocks, or incubators capable of 55°C and 70°C
- Shaking water bath capable of 55°C
- Vortexer
- 100% ethanol
- Isopropanol
- Optional: sterile deionized water
- Optional: Dithiothreitol (DTT)
- Optional: 3M NaOH

### Before Starting:

- Prepare DNA Wash Buffer and HBC Buffer according to the “Preparing Reagents” section on Page 4
- Set water baths, heat blocks, or incubators to 55°C and 70°C
- Heat Elution Buffer to 70°C

1. Place the swab in a 2 mL microcentrifuge tube.
2. Add 600  $\mu$ L TL Buffer and 20  $\mu$ L OB Protease Solution. Vortex at maximum speed for 30 seconds. If you are processing semen swabs, add 20  $\mu$ L DTT to each sample.
3. Incubate at 55°C for 60 minutes in a shaking water bath.

**Note:** If a shaking water bath is not available, vortex the sample every 10-20 minutes.

4. Briefly centrifuge to bring down any liquid from the top of the tube.
5. Add 620  $\mu$ L BL Buffer. Vortex at maximum speed for 20 seconds.



# Protocol for Swabs

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6. Incubate at 70°C for 10 minutes. Vortex at maximum speed for 10 seconds several times during incubation.
7. Briefly centrifuge to bring down any liquid from the top of the tube
8. Transfer the cleared lysate to a new 1.5 mL microcentrifuge tube (not provided).
9. Add 620  $\mu$ L 100% ethanol. Vortex at maximum speed for 15 seconds.
10. Briefly centrifuge to bring down any liquid from the top of the lid.
11. Insert a MicroElute® DNA Mini Column into a 2 mL Collection Tube.

## Optional Protocol for Column Equilibration:

1. Add 100  $\mu$ L 3M NaOH to the MicroElute® DNA Mini Column.
  2. Centrifuge at maximum speed for 30-60 seconds.
  3. Discard the filtrate and reuse the collection tube.
12. Transfer the sample from Step 10 (including any precipitate that may have formed) to the MicroElute® DNA Mini Column.
  13. Centrifuge at maximum speed for 1 minute.
  14. Repeat Steps 14-16 until all the sample has been transferred to the MicroElute® DNA Mini Column.
  15. Discard the filtrate and the Collection Tube.
  16. Transfer the MicroElute® DNA Mini Column to a new 2 mL Collection Tube.
  17. Add 500  $\mu$ L HBC Buffer.

**Note:** HBC Buffer must be diluted with isopropanol before use. Please see Page 6 for instructions.

# Protocol for Swabs

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18. Centrifuge at maximum speed for 30 seconds.

19. Discard the filtrate and reuse the Collection Tube.

20. Add 700  $\mu$ L DNA Wash Buffer.

**Note:** DNA Wash Buffer must be diluted with 100% ethanol prior to use. Please see the Preparing Reagents section on Page 4 for instructions.

21. Centrifuge at maximum speed for 1 minute.

22. Discard the filtrate and reuse the Collection Tube.

23. Repeat Steps 20-22 for a second DNA Wash Buffer wash step.

24. Centrifuge the empty MicroElute<sup>®</sup> DNA Mini Column at maximum speed for 2 minutes to dry the membrane.

**Note:** It is important to dry the column membrane before elution. Residual ethanol may interfere with downstream applications.

25. Transfer the MicroElute<sup>®</sup> DNA Mini Column to a nuclease-free 2 mL microcentrifuge tube (not provided).

26. Add 10-50  $\mu$ L Elution Buffer (or sterile deionized water) heated to 70°C.

**Note:** Smaller elution volumes will increase DNA concentration but decrease yield. Elution volumes greater than 200  $\mu$ L are not recommended.

27. Let sit at room temperature for 3 minutes.

28. Centrifuge at maximum speed for 1 minute.

29. Store DNA at -20°C.

# Protocol for Forensic Samples

## E.Z.N.A.<sup>®</sup> MicroElute<sup>®</sup> Genomic DNA Kit - Protocol for Forensic Samples

This protocol is designed for isolation of genomic DNA from forensic samples such as hair, cigarette butts, nail clippings, material stained with blood, saliva, or semen.

### Materials and Equipment to be Supplied by User:

- Microcentrifuge capable of at least 13,000 x *g*
- Nuclease-free 1.5 mL or 2 mL microcentrifuge tubes
- Water baths capable of 55°C and 70°C
- Shaking water bath capable of 55°C
- Vortexer
- 100% ethanol
- Isopropanol
- Optional: Dithiothreitol (DTT)
- Optional: sterile deionized water
- Optional: 3M NaOH

### Before Starting:

- Prepare DNA Wash Buffer and HBC Buffer according to the "Preparing Reagents" section on Page 4
  - Set water baths to 55°C and 70°C
  - Heat Elution Buffer to 70°C
1. Cut the sample to small pieces and place into a 2 mL microcentrifuge tube (not provided).
  2. Add 300  $\mu$ L TL Buffer. Vortex to mix thoroughly. If you are processing semen stains, add 20  $\mu$ L DTT to each sample.
  3. Add 20  $\mu$ L OB Protease Solution. Vortex at maximum speed for 30 seconds.
  4. Incubate mixture at 55°C for 60 minutes in a shaking water bath.

**Note:** If a shaking water bath is not available, vortex the sample every 10-20 minutes. Lysis time will depend on the amount and type of sample but is approximately one hour. Extend incubation time of hair samples until lysis is complete. Lysis can proceed overnight for larger samples of nail clippings.

# Protocol for Forensic Samples

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5. Briefly centrifuge to bring down any liquid from the top of the tube.
6. Add 320  $\mu$ L BL Buffer. Vortex at maximum speed for 20 seconds.
7. Incubate at 70°C for 10 minutes. Vortex at maximum speed for 10 seconds several times during incubation.
8. Centrifuge at maximum speed for 5 minutes.
9. Transfer the cleared supernatant to a new 2 mL microcentrifuge tube (not provided).
10. Add 320  $\mu$ L 100% ethanol. Vortex at maximum speed for 15 seconds.
11. Briefly centrifuge to bring down any liquid from the top of the lid.
12. Insert a MicroElute® DNA Mini Column into a 2 mL Collection Tube.

## Optional Protocol for Column Equilibration:

1. Add 100  $\mu$ L 3M NaOH to the MicroElute® DNA Mini Column.
  2. Centrifuge at maximum speed for 30-60 seconds.
  3. Discard the filtrate and reuse the collection tube.
13. Transfer the sample from Step 11 (including any precipitate that may have formed) to the MicroElute® DNA Mini Column.
  14. Centrifuge at maximum speed for 1 minute.
  15. Repeat Steps 13-14 until all the sample has been transferred to the MicroElute® DNA Mini Column.
  16. Discard the filtrate and the Collection Tube.

# Protocol for Forensic Samples

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17. Transfer the MicroElute® DNA Mini Column to a new 2 mL Collection Tube.

18. Add 500 µL HBC Buffer.

**Note:** HBC Buffer must be diluted with isopropanol before use. Please see Page 6 for instructions.

19. Centrifuge at maximum speed for 30 seconds.

20. Discard the filtrate and reuse the Collection Tube.

21. Add 700 µL DNA Wash Buffer.

**Note:** DNA Wash Buffer must be diluted with 100% ethanol prior to use. Please see the Preparing Reagents section on Page 4 for instructions.

22. Centrifuge at maximum speed for 1 minute.

23. Discard the filtrate and reuse the Collection Tube.

24. Repeat Steps 21-23 for a second DNA Wash Buffer wash step.

25. Centrifuge the empty MicroElute® DNA Mini Column at maximum speed for 2 minutes to dry the membrane.

**Note:** It is important to dry the column membrane before elution. Residual ethanol may interfere with downstream applications.

26. Transfer the MicroElute® DNA Mini Column to a nuclease-free 1.5 or 2 mL microcentrifuge tube (not provided).

27. Add 10-50 µL Elution Buffer (or sterile deionized water) heated to 70°C.

**Note:** Smaller elution volumes will increase DNA concentration but decrease yield. Elution volumes greater than 200 µL are not recommended.

# Protocol for Forensic Samples

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28. Let sit at room temperature for 3 minutes.
29. Centrifuge at maximum speed for 1 minute.
30. Store DNA at  $-20^{\circ}\text{C}$ .

## Troubleshooting Guide

Please use this guide to troubleshoot any problems that may arise. For further assistance, please contact the technical support staff, toll free, at **1-800-832-8896**.

Problem	Cause	Solution
Clogged Column	Incomplete lysis	Extend incubation time of lysis with TL Buffer and protease. Add the correct volume of BL Buffer and incubate for specified time at 70°C. It may be necessary to extend incubation time by 10 minutes.
	Sample too large	Do not use greater than the recommended amount of starting material. For larger samples, divide into multiple tubes.
	Incomplete homogenization	Pulverize material as indicated in liquid nitrogen to obtain a fine powder.
Low DNA yield	Clogged column	See above
	Poor elution	Repeat elution or increase elution volume. Incubate the column at 70°C for 5 minutes before centrifugation.
	Poor binding to column	Follow the protocol closely when adjusting the binding conditions.
	Improper washing	DNA Wash Buffer must be diluted with 100% ethanol before use.
HBC Buffer must be diluted with isopropanol before use.		
No DNA eluted	Poor cell lysis	Increase incubation time with TL Buffer. An overnight incubation may be necessary.
	Incomplete homogenization	Pulverize starting material as indicated in liquid nitrogen to obtain a fine powder.
	100% ethanol was not added before adding sample to column	Before applying DNA sample to column, add TL Buffer and 100% ethanol.
	Ethanol was not added to the DNA Wash Buffer	Dilute DNA Wash Buffer with the indicated volume of 100% ethanol before use.

## Ordering Information

The following components are available for purchase separately.  
(Call Toll Free at 1-800-832-8896)

Product	Part Number
RNase A, 5 mL	PD090
Proteinase K Solution, 10 mL	AC116
Elution Buffer, 100 mL	PDR048
DNA Wash Buffer, 100 mL	PS010

HiBind®, E.Z.N.A.®, and MicroElute® are registered trademarks of Omega Bio-tek, Inc.  
PCR is a patented process of Hoffman-La Roche. Use of the PCR process requires a license.